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Thermal Inactivation of Type E Botulinum Toxin[1]

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The theoretical required cooking times for inactivation of type E *Clostridium botulinum* toxin (5,000 LD_{50} mouse units per 0.5 ml) in haddock fillets of various sizes were calculated by graphical integration of the toxin inactivation rate and heat penetration data. The results indicated that normal cooking procedures should suffice to inactivate this amount of toxin. This conclusion was substantiated by the following additional experimental observations which revealed that the original experiments had been conducted under conservative conditions. First, maximal heat stability of the toxin was found to occur at about *p*H 5.5, with decreasing resistance upon increasing *p*H. The theoretical cooking times were based on destruction of the toxin at *p*H 6.7. The *p*H of radio-pasteurized inoculated haddock, when toxin production had occurred, was on the alkaline side, at which condition the toxin is heat-labile. Second, when spoilage was discernible in radio-pasteurized inoculated haddock, the toxin titer was low, about 50 LD_{50} mouse units per 0.5 ml. Third, the toxin was adequately inactivated in toxic fillets after deep-fat frying for 3 min at 375 F (190.6 C) or after pan frying for 5 min per side at 400 F (204.4 C). Fourth, in this study, residual toxin activity was assayed by intraperitoneal injection of mice. It was shown that the oral toxic dose was 50 to 100 times greater than the intraperitoneal toxic dose.

Radiation-pasteurization in conjunction with refrigerator storage has been shown to be feasible for extending the storage life of various marine products including haddock fillets. However, a deterrent to the immediate adoption of this process on a commercial scale has been posed by the studies of Ohye and Scott (7) and Schmidt, Lechowich, and Folinazzo (9), which indicated that *Clostridium botulinum* type E could grow and produce toxin at refrigerator temperatures above freezing. Furthermore, the radio-resistance of the spores of this organism is such that a significant degree of spore inactivation could not be expected from a radiation-pasteurizing dose (10).

Working with inoculated packs (100 or 10,000 spores of type E per gram) of radio-pasteurized haddock fillets held at various refrigerator temperatures, Goldblith and Nickerson (*unpublished data*) found that toxin production occurred only after the fillets had exceeded their expected shelf life at the particular storage temperature. However, when the inoculation was made at a level of 1 million spores per gram, the time at which the fillets became toxic coincided with the time at which the fillets were considered spoiled. A survey of freshly caught clams and haddock, and of commercially produced haddock fillets (Goldblith and Nickerson, *unpublished data*) indicated that the level of natural contamination with type E *C. botulinum* spores was considerably less than that used in the inoculated pack studies (maximum was found to be 0.17 spores per gram in only 20% of the samples tested).

In view of these findings, it is the opinion of some that the potential danger of botulism in radio-pasteurized haddock is not of practical importance; however, there are others who do not share this view because of outbreaks of botulism, type E, associated with seafood. Dolman (1) reported that, of the 36 known outbreaks of type E botulism to date, 29 of the cases were associated with the flesh of raw or improperly cooked fish; raw fish eggs, whale meat, or seal meat was implicated in six other cases. However, in no cases reported to date have commercially prepared fresh fish been implicated (only smoked or canned fish).

It is fortunate that in this country fish is cooked prior to eating except for a few specialty items.

[1] This is Contribution No. 968 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge.

However, this raises the question, is the cooking process adequate for inactivation of type E *C. botulinum* toxin?

The purpose of this investigation was to determine whether normal cooking methods would adequately inactivate type E *C. botulinum* toxin in radio-pasteurized haddock fillets, if the toxin was present initially.

MATERIALS AND METHODS

Type E *C. botulinum* toxin was produced by inoculating a spore mixture of *C. botulinum* strains 8E, Detroit, Beluga, and Minneapolis into Trypticase-peptone-glucose (TPG) broth and incubating for 3 days at 85 F (29.4 C). The cells were removed by centrifugation, and the cell-free toxic filtrate was stored at about 33 F (0.5 C) until used. On some instances a TPG-haddock broth was used for growing the toxin. In this case, the dehydrated ingredients were reconstituted with a haddock infusion prepared by simmering haddock fillets in distilled water (1 lb/liter) and filtering through glass wool.

Thermal inactivation studies of the toxin were carried out in the medium in which it was produced, that is, TPG broth or TPG-haddock broth. One particular study, however, was concerned with the thermal inactivation of the toxin in a haddock substrate. For this purpose, haddock fillets were comminuted and then were alternately frozen and thawed several times to rupture the cells and obtain some free-run cellular fluid. This fluid was heated to coagulate the soluble proteins, and then about 25% of the coagulum was added back to the heated fluid and dispersed by blending. This material served as the haddock substrate.

Specially made thin-wall glass thermal-death-time tubes were used in the thermal-inactivation studies. The approximate dimensions of the tubes were: inside diameter, 3 mm; wall thickness, 0.2 mm; and length, 150 mm. Portions (1 ml) of the toxic filtrate were placed into each tube by means of a hypodermic syringe fitted with a 15-gauge needle. A series of tubes were then heated for a prescribed time in a thermostatically controlled water bath which was constant to ± 0.15 F, and were immediately cooled in ice water. (Within the temperature range employed in this study, approximately 12 sec was required for the temperature at the center of the liquid column to reach 0.2 F below bath temperature.) The contents of a series of similarly heated tubes were extracted with a hypodermic syringe, pooled, and trypsinized for 45 min at 98.6 F (37 C). Portions (0.5 ml) of the toxic filtrate were injected intraperitoneally into groups of three mice (Swiss white, male, 18 to 25 g) for thermal-inactivation-time determinations, and into groups of six mice for LD_{50} assay. The mice were observed for 48 hr and then discarded. The thermal inactivation time was regarded as the time interval at a given heating temperature at which the break between death and no death occurred in groups of three mice injected with the heated toxin.

Quantitative counts for viable type E organisms in inoculated fillets were made by use of Miller-Prickett tubes and TPG agar to which was added, prior to pouring, 2.5 ml of a sterile 10% sodium bicarbonate solution per 150 ml. Tube cultures were incubated overnight at 85 F (29.4 C), and then for an additional 24 hr at 75 F (23.9 C).

To determine the total plate counts of haddock fillets, appropriate serial dilutions were plated on Eugonagar (BBL) enriched with yeast extract (5 g/liter) and incubated for 5 days at 68 F (20 C).

Toxic or fresh commercial fillets were prepared for cooking by coating with an egg batter and then rolling in cracker crumbs. The fillets were deep-fat fried in vegetable oil at 375 F (190.6 C) or pan-fried at 400 F (204.4 C) in a thermostatically controlled, deep, electric frying pan.

In determining the rate of heat penetration into a fillet during deep-fat frying, a thermocouple was inserted into the center of the thickest portion of the fillet, and instantaneous temperature measurements were recorded by means of a recorder with motor-driven chart. Heat penetration rates during pan frying were made on fish portions. These were manufactured commercially and had been cut to various uniform thicknesses from frozen fish blocks and then breaded.

Measurements of *p*H were made with a Beckman Zeromatic *p*H meter by immersing the electrodes in a 1:1 slurry of fish flesh and distilled water.

In assaying the cooked fillets for toxicity, each fillet was blended for 2 min in twice its weight of chilled gel-phosphate buffer (*p*H 6.2), allowed to stand for 1 to 2 hr at 50 F (10 C), centrifuged, trypsinized or not as the occasion required, and injected into mice intraperitoneally in 0.5-ml portions.

RESULTS

Thermal inactivation of type E toxin in a haddock substrate. The first objective of this investigation was to determine thermal inactivation times of type E botulinum toxin in a haddock substrate. For this purpose, 1 ml of a toxic filtrate of a mixed-strain type E *C. botulinum* culture grown in TPG broth was added to each 9 ml of a haddock substrate, the preparation of which is described in Materials and Methods. The initial titer of this toxic haddock substrate was 5,000 mouse LD_{50} per 0.5 ml.

Thermal inactivation times for the toxin were determined at temperatures ranging from 135 to 160 F (57.2 to 71.1 C), and these are shown plotted as a function of heating temperature (Fig. 1). Over the temperature range investigated, the thermal inactivation curve was found to be linear. Also, the thermal destruction of the toxin was strongly temperature-dependent as attested by the low Z value (negative reciprocal slope) of 7.5.

Heat penetration rate during cooking of haddock fillets. To determine the adequacy of the normal cooking process on the inactivation of type E toxin at this concentration (5,000 mouse LD_{50} per

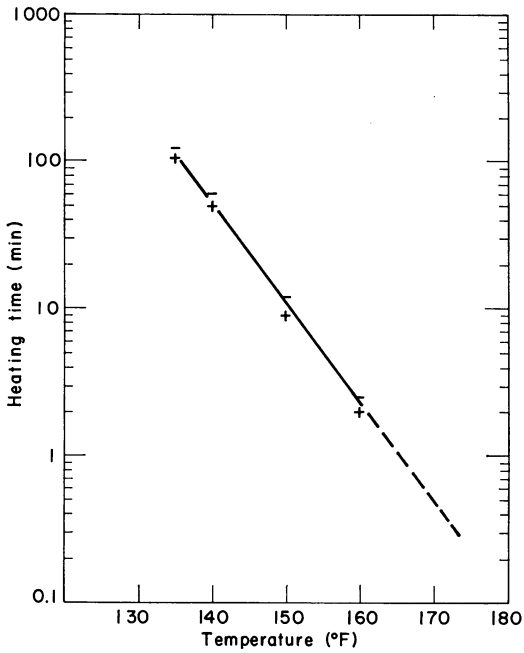

FIG. 1. *Thermal-inactivation-time curve for 5,000 LD_{50} per 0.5 ml of type E botulinum toxin in a haddock substrate. A plus sign indicates residual toxicity after heating for the particular time, that is, one or more deaths in the group of mice injected with the heated toxic filtrate. A minus sign signifies that no deaths resulted in the group of mice injected with the heated toxic filtrate, or there was no residual toxicity after heating for the particular time. The thermal-inactivation curve has been drawn in such a manner to lie above all the plus signs.*

0.5 ml) in haddock fillets, a knowledge of the rate of heat penetration into a fillet during cooking was required. This was determined in accordance with the following schedule: 0.5-inch (1.3-cm) thick fillets were deep-fat fried for 3 min; 0.75-inch (1.9-cm) thick fillets were deep fat fried for 5 min; 1-inch thick fillets were deep fat fried for 6.5 min; 0.5-inch thick fillets were pan fried for 4 min on each side; 0.75-inch thick fillets were pan fried for 5 min on each side. After cooking, the fillets were drained for several seconds and then were placed on an absorbent paper towel on a dish to cool. An organoleptic evaluation by a small panel had previously indicated that these particular cooking times resulted in the proper degree of "doneness" for the particular sized fillet. The rates of heat penetration are presented in Table 1. Each set of data shown represents the slowest heat penetration rate obtained during several replicate trials of a particular treatment. It should be noted that the maximal temperature attained at the center of the fillet does not occur at the end of the cooking period, but rather while the fillet is being drained.

Calculation of theoretical cooking time required to inactivate type E toxin. From the thermal-inactivation-time data and heat-penetration data, the "lethality" curves were obtained for the various cooking treatments. By graphical integration of the area under each "lethality" curve, the cooking times required to inactivate the toxin in the different sized fillets were calculated to be as follows.

(i) For deep fat frying: a 0.5-inch thick fillet would require a total time of 5.7 min (3-min cooking plus a 2.7-min draining period); a 0.75-inch thick fillet would require a total time of 6.7 min (5-min cooking plus a 1.7-min draining period); and a 1-inch thick fillet would require a total time of 11.4 min (6.5-min cooking plus a 5-min draining period).

(ii) Pan frying: for 0.5-inch thick fillets pan fried 4 min on each side, the toxin should be inactivated after 6.4 min of cooking time; for 0.75-inch thick fillets pan fried 5 min on each side, the toxin should be inactivated after 10.7 min (10 min of cooking plus 0.7 min of draining).

Since it is most likely that a fillet would not be eaten immediately after cooking, but, for one reason or another, not until at least 5 min had elapsed, it is felt that each of the cooking times employed for the different sized fillets would be adequate for inactivating type E *C. botulinum*

TABLE 1. *Rate of heat penetration into haddock fillets of different thicknesses during deep-fat frying at 375 F or pan frying at 400 F*

Time	Temp (F) of fillet				
	Deep-fat fry			Pan fry	
	0.5 inch	0.75 inch	1 inch	0.5 inch	0.75 inch
min					
0	50	42	48	40	39
1	71	47	53	53	42
2	106	64	64	80	48
3	146[a]	87	77	102	58
4	159	109	90	119[b]	67
5	161	135[a]	102	140	78[b]
6	161	167	116	166	94
6.5	160	172	124[a]	—	107
7	159	175	134	181	118
8	157	178	146	190[a]	138
9	155	178	154	194	151
10	153	178	158	192	162[a]
11	—	—	161	—	167
12	—	—	162	—	167

[a] End of cooking process.
[b] Fillet turned over.

toxin if present in the fillet at a concentration of 5,000 mouse LD_{50} per 0.5 ml.

Effect of toxin titer on thermal inactivation time. The next question that arose was whether these cooking times would be adequate if the toxin concentration were greater than 5,000 mouse LD_{50} per 0.5 ml. The next objective, therefore, was to determine the effect of thermal inactivation times as a function of toxin concentration. For this study, a toxic filtrate with a very high titer was desired so that the experiment could be conducted over a wide range of concentrations; however, attempts to produce a titer exceeding 50,000 mouse LD_{50} by varying the culture media or the incubation temperature were not successful. Thus, the cells were grown in a dialysis bag immersed in culture media (8), and by this method a toxic filtrate was obtained with a titer of 5,000,000 mouse LD_{50} per 0.5 ml. The medium outside of the bag was nontoxic, which indicated that the toxin molecules did not pass through the pores of the cellophane dialysis bag. This toxin proved to be very unstable. When the toxin was diluted by a factor of 10 in fresh TPG broth or in haddock substrate, there actually was a 500-fold reduction in potency after trypsinization. If the toxin was trypsinized first and then diluted, however, this reduction in toxin concentration did not occur. It would seem that, on diluting the toxin with fresh TPG broth or haddock substrate, the toxin molecules were aggregating or binding with other protein molecules in such a manner that trypsin activation was hindered.

Consequently, the experiment was repeated, with the exception that this time a haddock broth was used in place of distilled water to dissolve the solid medium. A toxin with a titer of 500,000 mouse LD_{50} per 0.5 ml was obtained in this manner, and, when this toxic filtrate was diluted with fresh TPG-haddock broth medium or haddock substrate, the dilution factor came out as expected (no loss of toxin except by dilution).

Decimal dilutions of this toxic filtrate were made with fresh TPG-haddock broth medium buffered to *p*H 7, and the thermal inactivation times for the different strengths of toxin were determined at 150 F (65.6 C). A plot of log inactivation time as a function of log toxin concentration is shown in Fig. 2. From the slope of the line, it can be determined that, for a 10-fold increase in toxin titer, the thermal-inactivation time increased approximately 5.5 times. Segner, Schmidt, and Boltz (*unpublished data*) found a 10-fold increase in heating time with a 10-fold increase in toxin titer over a concentration range of 1,000 to 20,000 MLD. However, these investigators carried out their experiments in phosphate buffer at *p*H 6.0, and the difference may be due either to substrate or to *p*H.

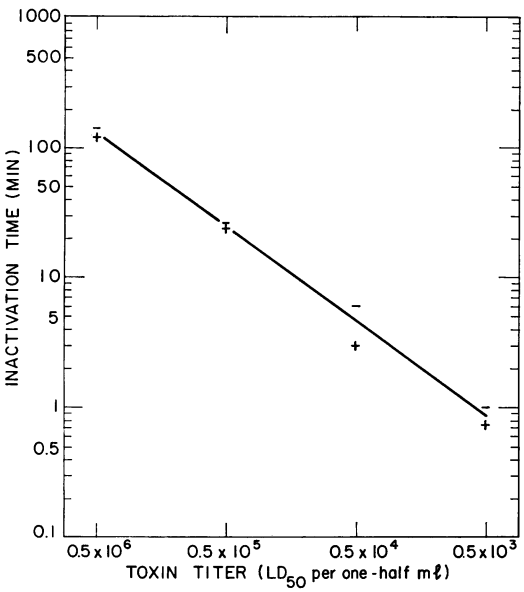

FIG. 2. *Effect of toxin titer on thermal inactivation of type E botulinum toxin at 150 F in TPG-haddock broth.*

Amount of toxin produced in haddock fillets by type E botulinum. Having determined the thermal-inactivation-time curve for the toxin in a haddock substrate and having determined the relation between toxin concentration and inactivation time, the next important consideration was the amount of toxin that could be produced by type E botulinum in haddock. For purposes of this investigation, portions of haddock fillets were inoculated with a spore mixture of type E *C. botulinum* (Detroit, 8E, and Minneapolis), irradiated with a dose of 200,000 rad of γ rays, and then stored at either 45 or 75 F (7.2 or 23.9 C). At various time intervals, a bacterial count was made of clostridia and a total plate count was made, and the fish flesh was assayed for toxicity. The results are presented in Table 2. At the storage temperature of 45 F, none of the fillets was toxic after 5 weeks, but at the end of 6 weeks some toxin production had occurred. The titer, however, was rather weak (approximately 50 mouse LD_{50} per 0.5 ml). The fish was considered decomposed and inedible after 4 weeks, at which time the total plate count was in excess of 800 million per gram.

At the storage temperature of 75 F, the maximal toxin concentration formed was about 5,000 mouse LD_{50} per 0.5 ml, and this was present after

TABLE 2. *Bacterial count and toxicity of haddock flesh inoculated with type E botulinum spores, irradiated, and then stored at 45 or 75 F*

Storage time	Bacterial count		Toxin titer (LD_{50}/0.5 ml)
	Clostridia/g	Total aerobes/g	
Weeks at 45 F			
3	10^2	19×10^6	None
4	10^2	81×10^7	None
5	15×10^4	58×10^7	None
6	2×10^6	32×10^7	5×10^1
Days at 75 F			
0	5×10^3	54×10^1	None
1	10^3	50×10^2	None
2	10^4	79×10^6	None
3	13×10^6	30×10^7	5×10^1
4	27×10^6	69×10^7	1×10^2
5	70×10^6	46×10^7	5×10^2
6	50×10^6	40×10^7	5×10^2
7	40×10^6	50×10^7	5×10^3
10	20×10^6	58×10^7	5×10^3

7 days. However, the fish was regarded as spoiled after 3 days, at which time the total plate count was 300 million per gram, and the toxin titer was about 50 mouse LD_{50} per 0.5 ml. It is of interest to note that the nontrypsinized fillets were usually more toxic than the trypsinized fillets.

Thus, since thermal-inactivation times for the toxin were originally determined at a concentration of 5,000 mouse LD_{50} per 0.5 ml in haddock substrate, and since it appears that this amount of toxin is produced in haddock fillets only in an advanced stage of decomposition, it is felt that any cooking time based on the destruction of this amount of toxin should have a considerable factor of safety.

Effect of pH on thermal stability of type E toxin. The next variable to be investigated was the effect of *p*H on the thermal inactivation of the toxin. Toxin was produced in TPG-haddock broth, and the *p*H of the toxic filtrate was measured to be 6.15. The thermal inactivation time at 150 F for the toxin (trypsinized after heating at the *p*H used) was determined at this *p*H and also at *p*H 7.05 and 8.10; 40% sodium hydroxide was used to adjust the *p*H. A plot of thermal inactivation time as a function of *p*H is shown in Fig. 3. It is quite obvious that *p*H exerted a marked influence on the thermal stability of the toxin. However, the possibility arose that the apparent instability at *p*H 8 compared with *p*H 6 was not due entirely to a *p*H effect, but to an incomplete trypsinization of the toxin at *p*H 8, since Duff, Wright, and Yarinsky (3) reported that the optimal *p*H for trypsinization of type E toxin at 98.6 F was approximately 6. Consequently, it was decided to repeat the experiment, except this time to work with trypsinized toxin. A series of previous experiments had indicated that the thermal-inactivation-time curves for the prototoxin and trypsinized toxin were practically identical (*unpublished data* of the authors); therefore, it was believed that the effect of *p*H on thermal stability of trypsinized toxin would be comparable to that of the prototoxin. The toxic TPG-haddock broth filtrate was, therefore, trypsinized at *p*H 6.15, samples were adjusted to various *p*H values with either 6 N hydrochloric acid or 40% sodium hydroxide, heated for different times at 150 F, and then injected into mice. The result of this experiment is depicted in Fig. 3 as the bell-shaped curve. The toxin appears to be most heat-stable at about *p*H 5.5. Ohye and Scott (7) reported that type E toxin had its greatest heat stability at *p*H 4.5 to 5. Segner, Schmidt, and Boltz (*unpublished data*) found the toxin to be most heat stable in the *p*H range of 5.18 to 5.64. However, these latter workers did not investigate the effect of *p*H below 5.18. These slight differences could be explained by the work of Sommer and Sommer (11), who, in general,

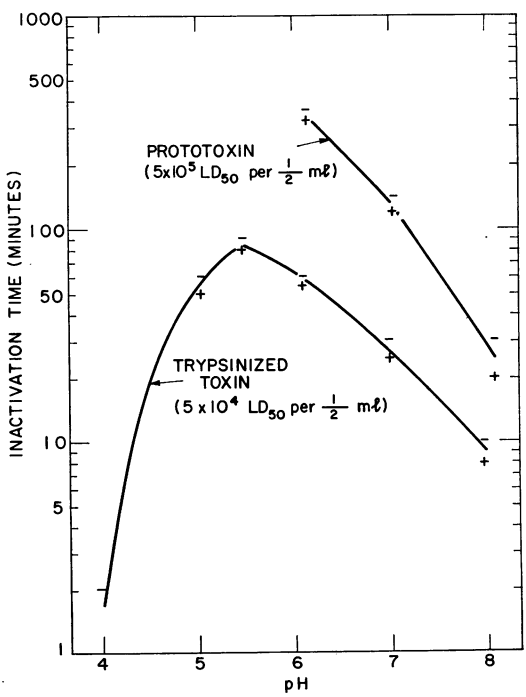

FIG. 3. *Effect of pH on thermal inactivation of type E botulinum toxin at 150 F in TPG-haddock broth.*

found the *p*H optimum for the stability of type A *C. botulinum* toxin to be at *p*H 5; however, they observed that the presence of foreign proteins could cause a slight shift in the optimal *p*H.

It can be observed that the curves for the prototoxin and trypsinized toxin converge slightly with increasing alkalinity over the *p*H range from 6 to 8. This is probably due to incomplete trypsinization of the prototoxin at the alkaline *p*H.

pH of haddock fillets at spoilage. It will be recalled that the original thermal-inactivation curve for the toxin was determined in a haddock substrate. The *p*H of this substrate was 6.7, and this is the approximate *p*H of fresh haddock. Since hydrogen ion concentration exerts a profound influence on the thermal inactivation time of the toxin, it becomes of extreme importance to know the *p*H of haddock flesh after type E *C. botulinum* has grown and produced toxin.

Consequently, portions of haddock fillets were inoculated with a spore mixture of type E *C. botulinum*, irradiated at 10^5, 2×10^5, or 3×10^5 rad, and then stored at 45 F. The inoculum of spores was such that there would be about 1,000 spores per gram after irradiation treatment. The particular radiation doses were selected because they represent the dose range being used for the experimental radio-pasteurization of seafood, and the concomitant spoilage flora, which might influence toxin production, may differ between the fillets receiving the low dosage and those at the higher dosage. At periodic intervals, bacterial counts were made, the *p*H was measured, and the flesh was assayed for toxicity (Table 3). With increasing growth of the aerobic population, the *p*H shifted toward the alkaline side. The fillets were considered to be spoiled after about 20 days, at which time the aerobic count was in the hundreds of millions per gram. The spoiled fish had a slight ammoniacal odor which would indicate an alkaline *p*H. In this experiment, the fillets were not toxic even after 30 days, at which time the *p*H was 7.4 to 7.5. It is believed, therefore, that when the fillets might have become toxic the *p*H would have been fairly alkaline. It is possible that toxin was being elaborated in small amounts, but was unstable at the alkaline condition.

Thus, in evaluating the adequacy of the cooking process for destroying type E *C. botulinum* toxin in haddock flesh, thermal-inactivation times were determined with slightly acidic conditions (*p*H 6.7), under which the toxin is more heat-resistant than at the usual alkaline condition of spoilage, and a toxin concentration was employed at a level much greater than would be present at the first detectable stage of spoilage. Both of these factors should represent a safety factor in the calculated cooking times.

TABLE 3. *Bacterial count, pH, and toxicity of haddock flesh inoculated with type E botulinum spores, irradiated, and then stored at 45 F*

Irradiation dose	Storage	*p*H	Bacterial count/g		Toxicity
			Clostridia	Aerobes	
rad	*days*				
100,000	7	7.0	35×10^3	80×10^4	Negative
	13	7.0	20×10^4	73×10^6	Negative
	20	7.05	5×10^4	80×10^6	Negative
	30	7.5	10×10^4	94×10^8	Negative
200,000	0	6.85	—	—	—
	7	6.5	6×10^3	52×10^4	Negative
	13	7.2	6×10^3	11×10^7	Negative
	20	7.1	60×10^3	61×10^7	Negative
	30	7.45	35×10^3	30×10^7	Negative
300,000	0	6.88	—	—	—
	7	6.55	12×10^2	30×10^4	Negative
	13	7.1	20×10^2	10×10^7	Negative
	20	7.15	10^2	90×10^7	Negative
	30	7.4	10^1	32×10^8	Negative

Cooking studies with toxic fillets. To confirm the validity of the calculated cooking times, it was decided to cook some toxic fillets for different times and then assay for residual toxicity. An attempt to obtain toxic fillets by injecting a toxic filtrate (TPG-haddock broth) into the fillets was unsuccessful because of the very low recovery of the injected toxin. Consequently, haddock fillets with a maximal thickness of 0.75 inch were inoculated with the Beluga strain of type E *C. botulinum*, sealed in Scotch-Pak bags, irradiated with 200,000 rad, and then held for 10 days at 75 F. Four random fillets were selected for an initial toxin assay, and the remainder were transferred to a refrigerator at 33 F. The toxin titer was found to be approximately 10,000 mouse LD_{50} per 0.5 ml for the nontrypsinized fillets, and 100 to 1,000 mouse LD_{50} per 0.5 ml for the trypsinized fillets. It is quite possible that the proteolytic enzymes of the fish flesh or of the aerobic bacterial flora may have activated the toxin, and further activation by trypsin resulted in a partial destruction. The *p*H of the toxic fillets was measured to be about 7.2.

The toxic fillets were removed from the refrigerator and cooked one at a time so as to maintain a low initial temperature. The cooking schedules were: for deep-fat frying, 3, 4, or 5 min at 375 F; for pan frying, 3, 4, 5, or 6 min on each side at 400 F. The deep-fat fry experiment

was carried out on duplicate fillets, and the pan fry experiment was performed on single fillets except for the 5 min per side cooking which was done on duplicate fillets. An important consideration was the length of time the fillets should be allowed to drain after cooking, since during this period a significant amount of destructive process occurs. It was believed that in actual practice this value would be about 5 min; however, to be conservative, it was decided to let the fillet remain in air for 3 min prior to plunging it into chilled buffer and comminuting to extract the toxin. The results of this study were as follows: none of the mice (in groups of three) injected with 0.5 ml of a 1:2 extract (trypsinized or nontrypsinized) of any of the cooked fillets died. A mouse in the group injected with the nontrypsinized extract of the 3 min per side pan-fried fillet, and also a mouse in the group shot with the nontrypsinized extract of the 4 min per side pan-fried fillet, showed symptoms (crusty eyes) of botulism but eventually recovered. All other mice appeared normal.

Comparison of oral toxic dose and intraperitoneal toxic dose. Throughout this investigation, toxicity was assayed by intraperitoneal injection of mice. However, since one is really concerned with residual toxin in a food that is to be ingested orally, it is of practical interest to determine the relation between the intraperitoneal toxic dose and the oral toxic dose. For type A botulinum toxin, Lamanna (4) stated that the oral dose for mice was about 50,000 times larger than the intraperitoneal dose. Dolman and Murakami (2) reported that for monkeys the ratio of subcutaneous to peroral minimal lethal dose of type E toxin was about 1:10. These authors did not state whether this applied to the trypsinized or the nontrypsinized toxin.

For this reason, an experiment was conducted to determine the difference between the intraperitoneal toxic dose and oral toxic dose for type E *C. botulinum* toxin. A toxic TPG-haddock broth was used as the test medium. Decimal dilutions of the test medium were made with gel-phosphate buffer and 0.5-ml portions of trypsinized or nontrypsinized solutions were either injected intraperitoneally into groups of six mice, or force-fed by use of special intubation needles modified for feeding infant rats (6). With the trypsinized toxin, the oral LD_{50} dose lay somewhere between the 1:100 and 1:1,000 dilution, and the intraperitoneal LD_{50} dose lay between the 1:10,000 and 1:100,000 dilution (Table 4). Thus, for the trypsinized toxin the oral LD_{50} dose was approximately 100 times the intraperitoneal LD_{50} dose. In the case of the nontrypsinized toxin, an approximate LD_{50} oral dose was arrived at by use of the undiluted toxic filtrate, whereas the intraperitoneal LD_{50} dose lay between the 1:10 and 1:100 dilution. Thus, for the nontrypsinized toxin the oral LD_{50} dose was approximately 50 times the intraperitoneal dose.

TABLE 4. *Comparison of the oral toxicity and intraperitoneal toxicity of type E botulinum toxin in mice*

Route	Dilution factor	No. of deaths/group of six mice	
		Trypsinized	Nontrypsinized
Oral	10^0	6/6	2/6
	10^{-1}	6/6	0/6
	10^{-2}	4/6	0/6
	10^{-3}	2/6	0/6
Intraperitoneal	10^0	—	6/6
	10^{-1}	—	6/6
	10^{-2}	6/6	0/6
	10^{-3}	6/6	0/6
	10^{-4}	5/6	—
	10^{-5}	1/6	—

The lethal oral dose was about 50 to 100 times greater than the lethal intraperitoneal dose. This fact represents still another safety factor in the determination of adequate cooking procedures for destroying type E botulinum toxin, since the intraperitoneal method of assay was used in the present investigation. However, this presupposes that the human is not more sensitive than mice to type E botulinum toxin. What little information is available seems to bear out this assumption (4).

All of the evidence obtained in the work herein reported, as well as that in the cited literature, indicates that radio-pasteurization of haddock fillets does not constitute a type E botulism public health hazard because: (i) if any toxin were present, it would be inactivated in properly cooked fillets because of the heat-labile character of the toxin; (ii) when spoilage of a radiopasteurized fillet is discernible, the level of toxin, if any, would be very low compared with the test dose used in this study; (iii) the *p*H of a radiopasteurized haddock fillet at spoilage is alkaline, a condition which reduces the thermal stability of the toxin; and (iv) the oral toxic dose is 50 to 100 times the intraperitoneal toxic dose, the latter dose being used to assay toxicity in this study.

In addition to the above, a bacterial survey of East Coast ground fish, to which a radio-pasteurization process may be applied, has indicated

that when this type E botulinum is present in these marine products the number of cells present is of such a low order that it is questionable as to whether or not outgrowth and toxin production would occur even under conditions of mishandling of the irradiated product (Goldblith and Nickerson, *unpublished data*).

At high levels of inoculation (10^4 per gram), tests in which irradiated and nonirradiated marine products have been compared have shown that the nonirradiated (inoculated control) product becomes toxic before toxin develops in the radiation-pasteurized fish (Goldblith and Nickerson, *unpublished data*).

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